REMARKS

Claims 1-15 are pending in this application; claims 9-15 are withdrawn from consideration. In view of at least the following remarks, reconsideration and allowance are respectfully requested.

I. Interview Summary

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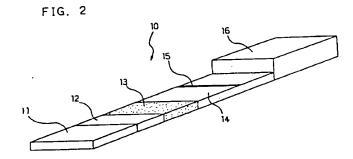
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(54) Immunochromatography-assisted device

(57) An immunochromatography-assisted device is disclosed which facilitates accurate and rapid detection of an antigen contained in a fluid sample and from which background coloring and blank coloring possibly causing a malfunction at detection are successfully eliminated. The immunochromatography-assisted device comprises a porous carrier (10) having an additive-impregnated part between a sample introducing part (11) and a determining part (14), the additive-impreg-

nated part (12) carrying at least one selected from the group consisting of a surfactant, a water-soluble ammonium salt and a pH buffer such that it dissolves in a sample introduced into the carrier (10) in order to move with the movement of the sample through the carrier (10). The porous carrier (10) also has a labelled part (13) segmented into plural zones.



Description



BACKGROUND OF THE INVENTION

[0001] The present invention relates to an extracorporeal diagnostic drug or a portable diagnostic device for detecting microorganisms in water and a trace amount of markers in the humor. More specifically, the present invention is intended to improve the performance of an immunochromatography-assisted device which facilitates simple and rapid measurements of such substances at small-scale clinics with no installation of analytical equipment and by the subjects studied.

[0002] Extracorporeal diagnostic drugs for detecting a trace amount of markers in the body fluids such as urine, sweat and blood have recognized importance as the major aids for clinical diagnosis and home care medicine. Immunochromatography is a basic technology for extracorporeal diagnostic drugs which best use specific reaction of an antibody. Among them, the most typical and widely applied immunochromatography-assisted device in the market as a general medicine is pregnancy testing drug. When pregnant, women secret human chorionic gonadotropin (hCG) from the placenta, which is later excreted in urine. Upon introduction of a female urine sample, a pregnancy tester determines whether the urine is positive or negative for pregnancy usually within 3 minutes or so, facilitating easy evaluation of pregnancy by any non-skilled ordinary person. A representative structure of such immunochromatography-assisted device is disclosed in the U.S. Patent No. 5,602,040, for example.

[0003] In the following, the structure and operation of a most simple immunochromatography-assisted device will be described, taking the pregnancy tester as an example. FIG. 1 illustrates the structure of a porous carrier for use in a typical immunochromatography-assisted device. The most widely used porous carrier is a sheet of nitrocellulose because the material readily allows fixation of an antibody. However, if the porous carrier 1 is formed with this material entirely, the flow rate of a sample reduces prominently. Therefore, it is preferable for the carrier to be formed with nitrocellulose only for a determining part and with a glass filter which allows a sample to flow more rapidly for the rest. A sample introducing part 2 located at one end of the carrier is made of a porous carrier such as glass filter that inherently absorbs a sample rapidly but is weak in retaining the sample, thus permitting a rapid and smooth passage of the sample toward a reaction-related part. If the porous carrier 1 is to be accommodated in a hollow case, the sample introducing part 2 is formed relatively long so as to partially expose it from the case to allow direct introduction of a urine sample into the sample introducing part 2. Alternatively, if the entire sample introduced into the sample introducing part.

[0004] A labelled part 3 is formed by impregnating a glass filter with an aqueous solution of an anti-hCG antibody which is adsorbed on colloidal gold or colored latex, followed by freezing and drying it. A determining part 4 is produced by dropping an aqueous solution of another antibody on a nitrocellulose sheet in an arbitrary shape, followed by drying and rinsing it. The very strong non-specific adsorption property of nitrocellulose ensures tight adhesion of the antibody to the nitrocellulose sheet. After fixation of the antibody, the nitrocellulose sheet may be surface-treated with bovine serum albumin (hereinafter abbreviated to "BSA"), for example, in order to prevent coloring of the background due to the non-specific adsorption property of nitrocellulose during antigen-antibody reaction for detection. This surface treatment is called blocking. A fluid absorbing part 5 located at the other end of the porous carrier is formed with a material such as glass filter which is excellent in fluid absorption and fluid absorbing capacity, for the purpose of rapid absorption of excess sample.

[0005] As a sample, female urine is introduced into the sample introducing part 2 in an amount as determined based on the shape of the immunochromatography-assisted device (normally 0.1 to 2 ml). The sample passes through the determining part 4 via the labelled part 3 toward the fluid absorbing part 5. When the sample passes around the labelled part 3, the labelled antibody which is carried on the labelled part 3 dissolves in water in the sample and is transferred toward the determining part 4 together with the sample. Subsequently, the labelled antibody dissolved in water arrives at the determining part 4. At that time, if the urine is positive for pregnancy, the labelled antibody reacts with the fixed antibody present at the determining part 4 via an antigen in the urine sample based on the specific antigen-antibody binding reaction, which causes the determining part 4 to be colored. If negative, such reaction would not occur and the labelled antibody uneventfully passes the determining part 4 to be absorbed in the fluid absorbing part. In this way, the presence or absence of an antigen in an aqueous sample solution can be determined.

[0006] In such immunochromatography-assisted device as described above, coloring of the part other than the antibody in a fixed phase present at the determining part (background coloring) and coloring of the antibody in the fixed phase in the absence of an analyte (blank coloring) reduce the S/N ratio during detection and become a factor for causing a malfunction. Background coloring is caused by a hydrophobic bond between a visualized antibody in a mobile phase and the porous carrier. On the other hand, blank coloring is caused by electrical interaction between the negative-charged antibody in the mobile phase and the positive-charged antibody in the fixed phase.

[0007] Normal samples for use in detection are free of such substance as surfactant, pH buffer or the like that offsets a hydrophobic bond and electrical interaction. Therefore, the conventional immunochromatography-assisted device has



drawbacks of development of not a little background coloring and occasional development of blank coloring. These drawbacks are particularly prominent when the antibody in the mobile phase is visualized with an organic dye.

[0008] Such immunochromatography-assisted device can be improved for its detectability by increasing the amount or concentration of the labelled antibody to be carried on the labelled part. However, this method disadvantageously increases the time until visual confirmation of background decoloring by sufficient depletion of the labelled antibody. In other words, an improvement in detectability contradicts a reduction in reaction time. If the antigen contained in a sample is over excess, the overall antibody in the mobile phase has to participate in antigen-antibody reaction, causing depletion of the mobile phase antibody at the determining part, which results in a malfunction of the device such that the amount of antigen is small seemingly. This is why a dynamic range in which reaction takes place essentially is preset in immunochromatography. The dynamic range for developing reaction is generally 50 to 100,000 IU/L with respect to the pregnancy tester.

[0009] Moreover, if an immunochromatography-assisted device is configured so as to ensure movement of a sample toward the determining part via the labelled part, hCG reacts with the labelled antibody before it reacts with the fixed antibody. Such structure is disadvantageous in detecting a low concentration of antigen as an analyte.

BRIEF SUMMARY OF THE INVENTION

[0010] In view of the above-mentioned drawbacks of the prior art device, the object of the present invention is to provide an immunochromatography-assisted device facilitating accurate and rapid detection of an antigen contained in a liquid sample by eliminating coloring of the background and blank coloring which may cause a malfunction during detection.

[0011] Another object of the present invention is to provide an epoch-making immunochromatography-assisted device for realizing improved detectability and reduced reaction time at the same time, and further for achieving an extended dynamic range for reaction.

[0012] In order to solve the above-mentioned problems, the present invention provides an immunochromatographyassisted device comprising a porous carrier having an impregnated part with an additive located between a sample introducing part and a determining part of the porous carrier, wherein the additive-impregnated part carries thereon at least one selected from the group consisting of a surfactant, a water-soluble ammonium salt and a pH buffer such that it dissolves in a sample introduced into the carrier in order to move with the movement of the sample through the carrier. The present invention also provides an immunochromatography device, wherein the labelled part is seg-

mented into plural zones.

[0014] The present invention provides a further immunochromatography-assisted device comprising a test strip composed of a porous carrier which has a labelled part containing one of two antibodies and a determining part containing the other of the two, the labelled part and the determining part being arranged such that a sample introduced into a sample introducing part of the porous carrier is allowed to move toward the determining part via the labelled part, wherein the porous carrier further comprises a bypass for the sample extending from the sample introducing part toward the determining part by passing through a periphery of the labelled part or by escaping the labelled part on the

[0015] While the novel features of the invention are set forth particularly in the appended claims, the invention, both as to organization and content, will be better understood and appreciated, along with other objects and features thereof, from the following detailed description taken in conjunction with the drawings.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING

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FIG. 1 is a perspective view illustrating a test strip of a conventional immunochromatography device.

FIG. 2 is a perspective view illustrating the structure of a test strip for use in an immunochromatography-assisted device in accordance with the present invention.

FIG. 3 is a perspective view illustrating another structure of the test strip for use in the immunochromatographyassisted device in accordance with the present invention.

FIG. 4 is a vertical cross-sectional view showing a test strip for use in one example of the present invention.

FIG. 5 is a graph illustrating the relationship between the concentration of hCG in a sample and the absorbance (coloring intensity) at the determining part.

FIG. 6 is a graph illustrating the relationship between the concentration of hCG in a sample and the minimal detectable time.

FIG. 7 is another graph illustrating the relationship between the concentration of hCG in a sample and the absorbance at the determining part.

- FIG. 8 is another graph illustrative relationship between the concentration of hCd sample and the minimal detectable time.
- FIG. 9 is still another graph illustrating the relationship between the concentration of hCG in a sample and th absorbance at the determining part.
- FIG. 10 is still another graph illustrating the relationship between the concentration of hCG in a sample and the minimal detectable time.
 - FIG. 11 is a graph illustrating the relationship between the concentration of hCG in a sample and the absorbance at the determining part obtained by varying the pH of a sample solution carried on the test strip.
 - FIG. 12 is a vertical cross-sectional view illustrating a test strip for use in another example of the present invention.
 - FIG. 13 is a vertical cross-sectional view illustrating a test strip for use in a further example of the present invention.
 - FIG. 14 is a graph illustrating the relationship between the concentration of hCG in a sample and the absorbance at the determining part.
 - FIG. 15 is a graph illustrating the relationship between the concentration of hCG in a sample and the minimal detectable time.
- FIG. 16 is another graph illustrating the relationship between the concentration of hCG in a sample and the absorbance at the determining part.

DETAILED DESCRIPTION OF THE INVENTION

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- [0017] The immunochromatography-assisted device in accordance with the present invention detects an antigen in an aqueous sample solution based on a specific antigen-antibody binding reaction, which comprises at least two different antibodies binding to an antigen as an analyte and a test strip composed of a porous carrier on which a labelled part containing one of the antibodies and a determining part containing the other of the antibodies are arranged such that a sample introduced into a sample introducing part is allowed to move toward the determining part via the labelled part, wherein one of the antibodies (the antibody in a mobile phase) is chemically visualized and contained in the labelled part of the porous carrier such that it dissolves in a sample solution introduced into the porous carrier in order to move inside the carrier with the movement of the sample solution, the other of the antibodies (the antibody in a fixed phase) is fixed to the determining part of the porous carrier to a degree not to be shifted in position by the movement of the sample, and the porous carrier has an impregnated part with an additive at a certain part between the sample introducing part and the determining part, the additive-impregnated part movably carrying thereon at least one selected from the group consisting of a surfactant, an ammonium salt and a pH buffer such that it dissolves in the sample solution and moves with the movement of the sample through the porous carrier.
 - [0018] The additive-impregnated part may be positioned between the sample introducing part and the labelled part or may be integrally combined with the sample introducing part.
- [0019] If the additive-impregnated part is positioned between the sample introducing part and the determining part, the additive such as surfactant, ammonium ion of the ammonium salt or pH buffer is mixed with an introduced sample and moves toward the determining part in the course of analysis. The sample is absorbed in a fluid absorbing part after arriving at the determining part.
- [0020] Of the various additives, the surfactant lowers the surface tension of a liquid sample to accelerate the flow of the sample, and modifies the surface characteristic of the antibody in the mobile phase to impair its hydrophobic bond with the porous carrier. This structure shortens the duration of detection with an immunochromatography-assisted device and inhibits coloring of the background to a minimum.
 - [0021] The ammonium ion, on the other hand, is a typical positive-charged compound, which effectively offsets the negative charge of the antibody in the mobile phase.
- 45 [0022] The pH buffer controls pH of the sample introduced, thereby offsetting the positive charge of the antibody in the fixed phase.
 - [0023] As such, these two additives can cancel electrical interaction between the mobile phase antibody and the fixed phase antibody, preventing the development of blank coloring.
 - [0024] In a preferred mode of the present invention, the labelled part is segmented into plural zones and a porous material for permitting movements of the sample and the labelled antibody is supplementarily positioned as a spacer between the plural zones of the labelled part such that the sample can flow through the segmented labelled part. The labelled part of this structure may carry different kinds and/or different absolute amounts of labelled antibody.
 - [0025] The labelled part may be segmented into plural zones such that plural zones of the segmented labelled part that are located proximal to the determining part, that is, those located downstream from the flow route carry the labelled antibody in a relatively large amount and plural zones that are located distal to the determining part, that is, those upstream from the flow route carry it in a small amount. This structure of the labelled part permits coexistence of a large amount of antigen in a sample with a large amount of labelled antibody in the labelled part downstream from the flow route if the sample contains a large amount of antigen. Thus, intense coloring can be induced in an acute phase



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of antigen-antibody reaction without developing a deficiency of the labelled antibody. If the sample contains only a small amount of antigen, the large amount of labelled antibody present downstream from the flow route fails to contribute to the antigen-antibody reaction; however, the antigen is condensed at the determining part and colored by the labelled antibody which later catches up with the antigen over time while flowing almost homogeneously. This means that this structure secures a high detectability of the device. In the latter case, since the labelled antibody which later catches up with the antigen has a low concentration, the background coloring remains diminished, causing no effect on the coloring of the determining part. According to this structure, an optimal reaction system participates in the antigen-antibody reaction, depending on the concentration of the antigen, which improves the dynamic range of detectability.

Division of the sample flow route into 2 routes is an effect produced by the bypass route extending from the sample introducing part toward the determining part such that the sample is allowed to partially pass the labelled part or to escape the labelled part to directly reach the determining part. One is a simple route that runs from the sample introducing part toward the determining part via the carrier. This route has minimal hindrances, and can pass the sample at a high rate. The alternative route passes through the labelled part, more preferably segmented labelled part, and develops contact-induced resistance on the interface between the labelled part and the spacer so as to delay the flow of the sample. In other words, according to this structure, upon introduction of a sample into the device, it occurs substantially that only the sample reaches the determining part and after a lapse of certain time the labelled antibody which has dissolved in the sample solution catches up with the sample to finally reach the determining part. During the delayed lapse of time, the determining part preliminarily accepts a mixture of the labelled antibody and the antigen in the sample such that the antigen is bound to the labelled antibody to a certain degree. Thus, the antigen can be captured efficiently even when the antigen in the sample is small in amount, resulting in improved detectability of the device. This structure appears to prolong the reaction time seemingly due to the presence of a delayed lapse of time. However, from the fact that the detectability of the device can be improved substantially without increasing an absolute amount of the labelled antibody carried on the labelled part, the time required for diminishing the background coloring can be reduced, which shortens the overall reaction time as a whole.

[0027] In another preferred mode of the present invention, the chemically visualized antibody, that is, mobile phase antibody is prepared by binding the antibody to any one of colloidal gold, latex particle, organic dye, pigment, metal, metallic oxide and enzyme or an arbitrary combination of a plurality of the above group to visualize the antibody. One example of preferred dyes is cyanine dye represented by the following chemical formula:

[0028] The immunochromatography-assisted device in accordance with the present invention is particularly effective if the antigen is either hCG, luteinizing hormone (LH) or C-reactive protein (CRP).

[0029] The surfactant for use in the present invention may be exemplified as non-ionic alkyl phenol ether surfactant. A Triton surfactant, particularly commercially available Triton X-100 is preferred.

[0030] Preferred ammonium salts include tetramethyl ammonium salts exemplified as tetramethyl ammonium chloride, tetramethyl ammonium iodide, etc.

[0031] It is preferable for the additive-impregnated part to carry a pH buffer in such an amount that makes a pH of 8.0 to 8.5 for a sample at the determining part when the sample is introduced into the sample introducing part of the carrier in a predetermined volume.